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EXPERIMENTS ON COLOR-VISION OF THE HONEY BEE.

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INTRODUCTION.

Whether insects can or cannot distinguish colors is a matter of much theoretical importance, for the correct interpretation of the relation of insects to flowers depends upon this answer. Most students of natural selection believed, at one time, that the forms and colors of flowers were adaptations to insect visitors. Lately there has been a reaction based on the general consensus of opinion, among morphological entomologists, concerning the poorness of insect vision. Kellogg[1] writes: "The fixed short focal distance, the incompleteness and lack of detail incident to a mosaic image, and the lack of accommodation (only partly provided for by the shifting of the peripheral pigment) to varying light intensity, which are admitted conditions of insect vision, make it seem difficult to account for the intricacy in pattern common to many flowers on a basis of adaptation to animal visitors of such poor seeing capacity as insects.

"Experimental evidence touching this criticism is singularly meager when one considers the importance of the subject. If insects can accurately distinguish colors, and at some distance, and can perceive the fine details of color-pattern at a very short distance, then the explanation of floral structure and pattern as adaptation to insect visitors has solid foundation for even the amazingly large and varied results which it attempts to explain; if not, it is hard to understand how the explanation is valid (at

[1]Kellogg, V. L., "American Insects," Henry Holt & Co., second edition, revised, 1908, pp. 580.

least in any such all-sufficient degree as commonly held), despite its logical character (in light of our knowledge of the nearly limitless capacity for modification of natural selection) and the abundant confirmatory evidence.

"Most of the experimental evidence so far offered is that included in Darwin's account ('On the Fertilization of Flowers by Insects'); in Lubbock's account of his experiments on honey-bees, familiar because of its presentation in his readable book, 'Ants, Bees and Wasps'; and in Plateau's account of his more recent but less familiarly known experiments with various insects including bees. Both Lubbock and Plateau are investigators ingenious in device, keen in deduction, and of unquestioned scientific honesty. Yet their conclusions are a direct contradiction. Lubbock believes that bees recognize colors at a considerable distance, that they 'prefer one color to another, and that blue is distinctly their favorite.' Plateau finds that neither the form nor the brilliant colors of flowers seem to have any important attractive rôle, 'as insects visit flowers whose colors and forms are masked by green leaves, as well as to continue to visit flowers which have been almost totally denuded of colored parts'; that insects show no preference or antipathy for different colors which flowers of different varieties of the same or of allied species may show; that flowers concealed by foliage are readily discovered and visited; that insects ordinarily pay no attention to flowers artificially made of colored paper or of cloth whether these artifacts are provided or not with honey, while, on the contrary, flowers artificially made of living green leaves and provided with honey are visited (from the attraction of the 'natural vegetable odor'). From these observations Plateau concludes that 'insects are guided with certainty to flowers with pollen or nectar *by a sense other than that of vision and which can only be that of smell*,' and finds particular proof of this in the facts, according to his observations, (1) that insects tend, without hesitation, towards flowers usually neglected by reason of the absence or poverty of nectar, from the moment that one supplies these flowers with artificial nectar, represented by honey; (2) that insects cease their visits when one cuts out the nectary without injuring the colored parts, and re-begin their visit if one replaces

the destroyed nectary by honey; (3) that it suffices to attract numerous insects if one puts honey on or in normally anemophilous flowers, simply green or brown in color, which are normally practically invisible and almost never visited by insects; and (4) that the visiting of flowers artificially made of fresh green leaves and containing honey demonstrates plainly the rôle of the sense of smell.

"It must be said that, despite many just criticisms that may be made on the character of his experiments, Plateau has made necessary more experimentation for the relief of the general theory that floral adaptation of color is due to color preferences of insect visitors."

Forel[1] and von Buttel-Reepen[2] are opposed to Plateau's views, but Bethe[3] is in accord with Plateau.

To test his conclusions, Forel repeated, in the following manner, Plateau's dahlia experiment. (1) Paper dahlias were distributed among some dahlias from which a large number of bees were collecting honey. The bees paid no attention to these artifacts. (2) Honey was placed on these artifacts, and, by skillful manipulation, brought to the attention of one of the bees. Immediately that bee neglected the real dahlias for these artificial ones. (3) Gradually all of the bees neglected the dahlias for those artifacts with their inexhaustible supply of honey—inexhaustible because it was constantly replenished by Forel. (4) The artifacts were removed. After a lapse of several days, similar artifacts, but containing no honey, were scattered among those dahlias. Immediately the bees neglected the dahlias for the artifacts, which they searched for honey. Forel thinks this experiment shows that bees have space, form and color perception.

Von Buttel-Reepen bases his opposition to Plateau's views largely upon information furnished him by Herr Roth, leader of the Baden bee-keepers school, and a teacher named Staehelin.

[1]Forel, Aug., "Die psychischen Faehigkeiten der Ameisen und einiger anderer Insekten," Muenchen, 1901. "Ants and Some of their Instincts," *Monist*, vol. 14, 1903–4.

[2]Buttel-Reepen, H. von, "Sind die Bienen Reflex-maschinen?, Experimental Beiträge zur Biologie der Honigbiene," *Biol. Centralbl.*, Bd. 20, 1900. "Are Bees Reflex Machines?" translated by Mary H. Geisler, Medina, O., 1907.

[3]Bethe, A., "Die Heimfähigkeit der Ameisen und Bienen zum Theil nach neuen Versuchen," *Biol. Centribl.*, Bd. 22, 1902.

Von Buttel-Reepen states: "We have seen above that the flight [of bees] becomes very unsafe in the dusk; therefore it is evident that gloomy weather influences considerably the ability to orient. 'One of my former neighbors,' Roth says in his communication, 'painted the gable of his house over the apiary with a sky-blue (luftblau) color. The same bees which always flew over the gable, on the next dark day, bumped against it with their heads, trying to fly through it.' A teacher, Staehelin, made the following observations: A weak after-swarm, mostly of young bees from a hive painted blue, dispersed among the masses of humming bees which were just taking their flight of orientation out of the other hives (which, as is usually the case in Germany, Switzerland, and Austria, were standing close together), and settled here and there in clumps. After a short time they flew back to the bee-house; but only a few found the right hive; the rest flew to other colonies, and to which? Only to those where a blue door invited them did they attempt an entrance, but nowhere else. Unfortunately they were so hostilely received that the ground in front of all of the blue hives was covered with bees."

Bethe had a swarm of bees lodged in a brown hive which rested on a table. He painted the outside of the hive blue and covered the table with green branches. Instead of the backgrond of trees, he substituted one of white and yellow flowered cloth. No change was produced in the home-coming of the bees. This Bethe considers conclusive proof that bees are not guided home by memory picture contributed by the eyes.

So far as my knowledge goes, M. Gaston Bonnier[1] is the only recent investigator who furnishes any experimental evidence that supports Bethe's view. He found that bees, the eyes of which had been rendered opaque with pigmented collodion, would pass direct to the hive from any distance less than three kilometers. This observation, which is not in harmony with Forel's experience,[2] supports Bethe's contention, but it has no direct bearing upon color vision.

The purpose of this paper is not to discuss the homing of the

[1]Bonnier, M. Gaston, "Le sens de la direction chez les abeilles," *C. R. Acad. Sci.*, Paris, T. CXLVIII., 1909, pp. 1019–1022.

[2]Forel always found that bees, the eyes of which had been rendered opaque, could not find their way home.

honey bee; but, by means of simple experiments, to throw some light upon the question "Can bees distinguish colors?"

Description of the Experiments.

The following experiments were performed in a large field just west of O'Fallon Park, St. Louis, Mo. The white sweet clover (*Melilotus alba* Lam.), with its long racemes of white papilionaceous flowers, was abundant in dense patches; but there were a few vacant places in the field. Foraging bees were visiting this white melilot in large numbers.

Series I. (July 12, 2 P.M.).

The discs used in this series of experiments were cut from colored cardboard, and each was six centimeters in diameter.

Experiment 1.—*I placed six discs of red cardboard on the top of rods that had been erected in the midst of a patch of white sweet clover. The rods were so adjusted that the top of each was about on a level with the tops of the weeds. Six similar discs were attached, at different heights, to the branches of the weeds. Honey was placed on all of these discs.*

More than an hour passed by and no response was made to these discs by the bees; but both flies and wasps visited them. The weeds were full of bees that were continuously flying to and fro in the immediate vicinity of these artifacts with their copious supply of honey. Were the odor of honey alone sufficient to attract bees reflexly, these bees should have been attracted early. After waiting half an hour, I decided to force the bees to attend to my artifacts.

Experiment 2.—*A bee was captured in a wide-mouth bottle and the bottle, with the cork removed, inverted over one of the red discs of experiment 1, until the bee dropped upon the disc; the bottle was then removed. This was tried with six different bees.*

In each case the bee always ascended to the top of the bottle and attempted to escape. After several futile efforts it would drop, either by accident or from exhaustion, upon the disc. At that moment, I always removed the bottle. Immediately the bee would leave never to return. Some of the bees fell into the honey; but, even in that case, they did not return.

EXPERIMENT 3.—*A branch containing blossoms on which a bee was foraging was gently removed from the plant and so manipulated that the bee was less than two centimeters from the honey of one of the discs of experiment 1. This was tried with six bees.*

In no case did the bee pay any attention either to the honey or to my discs. The bee always left immediately and went to one of the blossoms of the melilotus.

EXPERIMENT 4.—*Whenever a bee alighted on a blossom near one of the discs of experiment 1, I gently moved the sprig until the bee was brought to within less than two centimeters of the honey. This was tried with a dozen bees.*

No response was made to the honey.

In a cluster of weeds about a yard from the one in which most of my discs were located, I had placed, at the beginning of this series of experiments, a red disc so copiously supplied with honey that it overflowed upon the weed. This disc was so situated that by simply raising my eyes I could see it. Although the melilotus was swarming with bees, that disc remained in that place for nearly two hours before receiving its first visit from a bee. At that time, however, a bee hovered at the edge of the disc and began to sip the honey. It then alighted on the edge of the disc and continued to sip the honey. Almost immediately another bee flew up to this one. They both circled about for a moment and then alighted on the disc; one on the edge and the other near the center of the upper surface. From this time on, all of my attention was focused upon this plant.

EXPERIMENT 5.—*Near this disc was a blossom which I had wet with honey. While the two bees mentioned in the above experiment were foraging on disc one, a bee alighted on this blossom. I gently moved the sprig until the bee was within about a centimeter of the two bees just mentioned.*

It left the blossom and, alighting on the disc, began to forage.

EXPERIMENT 6.—*While these bees were imbibing honey, I attached two other red discs, each supplied with honey, to other branches of the weed.* (For descriptive purposes, starting with the disc upon which the bees were feeding, we will designate them disc one, disc two, disc three.)

One by one, the three bees on disc one departed for the hive.

On leaving, each hovered a moment above the disc, circled around it, made two or more short circles about the weed, then, ascending, departed. In about five minutes, two of the bees had returned to the weed. They did not visit any of the blossoms; but, after circling in the vicinity of the disc for a moment, alighted on the disc and began to imbibe the honey. They arrived less than half a minute apart. After securing a supply of honey, they departed in the same manner that they did before. In less than five minutes, the two had returned. One of these, after arriving at disc one, left it and went to disc two and obtained honey. These three bees were watched carefully for half an hour. In that time fourteen visits were made to the red discs; eleven to disc one and three to disc two. No visits were made to disc three. On two occasions three and on two other occasions two bees were on disc one at the same time. On each of these occasions, before departing for the hive, the bees always explored the neighborhood of the disc.

For the half hour or more that I was conducting this experiment, I was too much occupied to pay any attention to the discs on the other weed. I now watched them continuously for fifteen minutes. Although the weed was alive with bees, no bee visited the red discs. Had any of the bees discovered those discs and had they begun to collect honey from them, some of them should have made a return trip while I was watching. It is reasonable to conclude that no bees had visited them.

Thus, although over a dozen discs, well supplied with honey, were exposed, on weeds that were alive with bees, for fully three hours, yet only three bees visited those discs and their visits were confined to two of them![1]

EXPERIMENT 7.—*On leaving for home, all of the discs were removed from the field except the three from which the bees were collecting honey. On those discs I placed all of the honey that they would hold.* This was done hoping that, before dark, other bees, by imitation, would learn to collect honey from those discs.

[1] I have tried this same type of experiments at other times. Although considerable time always elapsed before the discs were attended to by the bees, yet the time was seldom as long as this.

Series II. (July 13, 8 A.M.)

On my arrival at the field this morning, I noticed that all of the honey had been removed from the discs; and, in two cases, that much of the color had been removed in spots. It looked as though the bees had attempted to carry off even the paper that had been saturated with honey.

EXPERIMENT 8.—*Among the branches of the same plants of melilotus from which, yesterday, a few bees learned to collect honey, I placed six red and six blue discs. Two of the red discs (1, 4) and one of the blue were attached to the tops of rods five feet high (the height of the weeds), the others were pinned, at different levels, to the branches of the weed. In the center of each red disc, honey was placed. The red discs were numbered from 1 to 6, the blue from 7 to 12. There was no honey on the blue discs.*

Almost immediately a bee alighted on disc one. These discs were watched continuously for a little less than a half hour. During that time no bees visited the blue discs, but they made thirty-nine visits[1] to the red discs. These visits were distributed as follows: disc one, seven; disc two, two; disc three, nine; disc four, seven; disc five, six; disc six, eight. The bees visited the discs on the rods (1, 4) just as readily as they did those attached to the weeds; they visited those high up (1, 2, 4, 6) just as frequently as they did those low down (3, 5). Whenever a bee was ready to depart for the hive, it always made, in the manner already described, a careful orienting flight. From now on the bees began to visit the red discs in such large numbers that it was impossible to keep an accurate record of the number of visits Sometimes as many as ten bees would visit the same disc at the same time.[2] The significance of this marked change in the behavior of the bees will be discussed later.

EXPERIMENT 9.—*I selected a red disc from which four bees had been collecting honey and, while the bees were away, placed it about six inches lower on the plant. In its place I placed a blue disc. The blue disc did not have any honey on it.*

[1]In all of these experiments, whenever a bee alighted on one of my artifacts, it was counted a visit, whether it was the arrival of a new bee or a return visit of a former visitor.

[2]When these experiments were first planned, it was my intention to mark each bee that participated; but, at this stage of the work, I realized that such a procedure would be impracticable and my paint and brush were put away.

The bees that arrived at the weed in the vicinity of the blue disc dropped at once to the red disc, without even pausing before the blue.

EXPERIMENT 10.—*I placed three more rods in the midst of the same weed; on two of them I placed blue discs without honey, and on the other one a red disc with honey.*

At the end of fifteen minutes, all of the red discs were being visited by numerous bees; none of the blue discs were being visited.

EXPERIMENT 11.—*Two rods were placed so near each other that a space of not more than two centimeters separated the discs. One disc was red and the other blue. The red disc was supplied with honey, the blue was not. After they had been in one position for fifteen minutes, the red disc was placed where the blue had been and the blue placed in the place from which the red had been taken.*

During the few minutes that these discs were under observation, many bees visited the red disc; on one occasion, three bees were obtaining honey from it at the same time. Only one bee visited the blue, and, evidently, she was not foraging for honey. She spent at least ten minutes on the disc and most of that time was spent in one place. A part of the time she was rubbing her legs against the edge of the disc, the remainder she seemed to be simply resting.

EXPERIMENT 12.—*Five centimeters from a red disc containing honey, I placed a blue disc containing honey.*

During the ten minutes that these discs were watched, twelve visits were made to the red disc and only one to the blue. On three different occasions, there were three bees on the red disc at the same time. It was at the close of the ten minutes that the first bee visited the blue disc.

Just as soon as the bee had imbibed the honey and left the blue disc, the disc was replaced with a blue disc that was not supplied with honey.

During the next five minutes, five visits were made to the red and none to the blue. One bee hovered momentarily above the blue and then went to the red.

EXPERIMENT 13.—*In a different place from that where experiment twelve was performed, I arranged, close together on rods, one blue*

and two red discs. On the blue disc and on one of the red discs honey was placed.

During the time these discs were under continuous observation, fifteen visits were made to the red disc that was supplied with honey, one bee alighted on the red disc that did not bear honey, and three bees alighted on the blue disc. These three bees visited the blue disc at the same time; one bee alighted on the disc, and then almost immediately the other two followed.

While the three bees just mentioned were on the blue disc, the rod supporting that disc was gently removed to a portion of the melilotus patch that did not contain any of my experimental discs.

One by one, the bees made a careful orienting flight and then flew away. These discs were no longer kept under continuous observation; but, at regular intervals, they were visited and the honey replenished. On those occasions I would watch each disc for about five minutes. On each trip I found three bees visiting the blue disc. The disc might be free from bees when I arrived; in a short time, however, three bees would arrive. They did not arrive simultaneously; but, before the first arrival had left two more would be there. I therefore concluded that the same three bees that discovered the honey on the blue disc had continued to visit it, and that no other bees had grasped the significance of that blue disc. (Unfortunately, for the reason mentioned above, these bees were not marked and one cannot be absolutely certain of their identity; but, from a knowledge of the habits of bees when foraging and of the time required to make a trip to the hive, I feel certain that they were the same bees.)

EXPERIMENT 14.—*While several bees were collecting honey from one of the red discs that capped one of my rods, the rod was gently carried fifteen feet in the direction of the hive and erected in another patch of melilotus.*

One by one, the bees made a careful orienting flight and then flew away. In a short time they had returned. Often eight or ten bees would be on the disc at the same time. While I was taking my notes, some of the bees hovered within a short distance of the small pad (13 × 8 cm.) on which I was writing, as though they were examining it. From now on this behavior was common.

EXPERIMENT 15.—*While ten bees were foraging on the red disc*

used in experiment fourteen, the rod was gently carried fifteen feet nearer the hive and erected in a place that was free from tall weeds; even the grass had been cropped short by a horse that had been grazing there.

The bees, on leaving, hovered about the disc a long time, even examining the cork to which the disc was pinned. Then, after describing a shallow spiral, they flew away. In a short time six had returned to this disc. On her return, each bee flew to the cork first and then to the top of the disc. I was now forced to leave the experiment for half an hour. On my return, the disc was being visited by numerous bees. During the five minutes that I watched it, twenty-six visits were made to it. There were always from six to ten bees on the disc.

EXPERIMENT 16.—*From the melilotus weed in which the first experiments of this series were performed, I gently removed a rod which was capped with a red disc upon which ten bees were foraging and erected it fifteen feet further away from the hive, in another patch of white sweet clover.*

The bees on leaving made a careful orienting flight. I left the disc for about twenty minutes. On my return, I found seven bees resting on the disc and imbibing honey.

EXPERIMENT 17.—*At that end of the field which was most distant from the hive, and at about fifty yards from the weeds in which the first experiments of this series were performed, there was a large patch of the same plants, from the racemes of which numerous bees were collecting honey. In the midst of these weeds, but well exposed, I placed a red disc upon which I had poured some honey.*

I remained by this rod for twenty minutes, but no bee approached it. At intervals of ten minutes, I made six visits to this disc. Each time I remained five minutes. At no time did I find any bees visiting the disc.

EXPERIMENT 18.—*In a space free from tall weeds, about thirty yards nearer the hive than the weed in which the first experiments of this series were performed, I erected one of my five foot rods and on its top placed a red disc well supplied with honey.*

At intervals of ten minutes, I made four visits to this disc. The first time I found one bee on the disc; the second time, three; the third time, two, and the fourth, four.

At this stage, I removed all of the discs from the field except the one used in this experiment.

At intervals of ten minutes, I made two additional visits to this disc. On the first trip I found ten bees on the disc; on the second trip, I found eight.

On leaving for home all of the discs were removed from the field.

Series III. (July 14, 7:30 A.M.)

Apparatus.—The cornucopias used in this series of experiments were made in the following manner: A piece of cardboard, colored on both sides, was cut the shape and dimensions shown in Fig. 1. It was folded along the dotted lines and the flaps

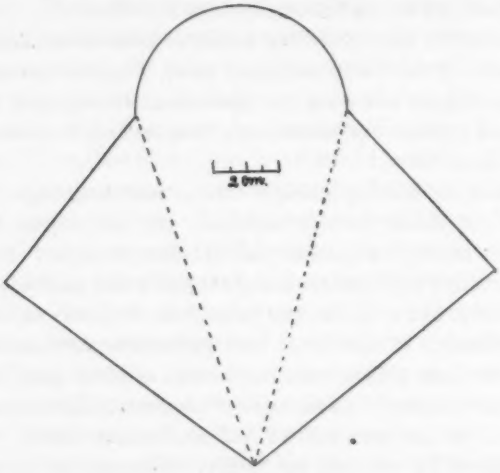


FIG. 1.

fastened where they lapped. About one centimeter of the apex of the cone was bent over and fastened. When finished, each cornucopia was nine centimeters long, with an elliptical lip six centimeters wide and three centimeters high. The lip was used for attaching the cornucopia to some support. Incidentally it furnished a platform for the bees. Some of the cornucopias were red and some were green.

EXPERIMENT 19.—*In the weeds that were the seat of the experiments of yesterday, several cornucopias were arranged at different*

levels. Half of these were red and half were green and they were arranged in pairs, one red and one green constituting a pair. The openings of both members of a pair faced in the same direction. All of these cornucopias were attached to parts of the weed. The red ones were supplied with honey, but the green were not.

Immediately after I had pinned the first red cornucopia to the bush, a bee entered it and began to collect the honey. Two of these pairs were kept under observation for nearly an hour. During that time numerous bees entered the red cornucopias, but not a bee entered the green ones.

EXPERIMENT 20.—*Five red cornucopias, each containing honey, were arranged on rods in the following manner: A, in a patch of melilotus about fifty yards further from the hive than the weeds that were the site of experiment nineteen; B, C, D, arranged in a line extending from the patch that was the site of experiment nineteen towards the hive, B twenty feet from the patch, C thirty-five feet, and D fifty feet; E sixty yards from the same patch mentioned above in a line which, at the experiment patch, made an angle of 45° with the line containing B, C, and D. Each of these cornucopias was visited once every twenty minutes, at which times it was watched closely for three minutes. The number of bees in the cornucopia when I arrived were counted, the number to arrive after I did were noted and the sum of the two numbers recorded.*

The results of the above experiment are recorded in the following table:

	Name of the Cornucopia.				
	A	B	C	D	E
Number of bees observed on trip 1	0	1	7	1	0
Number of bees observed on trip 2	0	8	2	8	0
Number of bees observed on trip 3	0	12	6	6	0
Number of bees observed on trip 4	1[1]	21	13	11	2[1]
Number of bees observed on trip 5	1	X	12	16	1
Number of bees observed on trip 6	0	X	X	X	0
Number of bees observed on trip 7[2]	1[3]	21	X	X	6

[1]Before leaving the bee made a careful orienting flight. She examined the cornucopia on all sides several times, reentered it three times, and, after describing a corkscrew curve, flew away to the hive.

[2]There was an interval of an hour between trip six and trip seven.

[3]Two other bees hovered near, but did not enter. The one that entered left immediately. The cornucopia was swarming with ants.

X means that the bees were so numerous that it was impossible to make an accurate count.

EXPERIMENT 21.—*Side by side, on one of the branches of the melilotus weed upon which most of these experiments were conducted, I arranged a red and a green cornucopia, and placed honey in each.*

During the first five minutes that these were under observation twenty-five bees entered the red cornucopia and three the green.

EXPERIMENT 22. *On rods erected in the open space between the experiment weed and the hive, and about three feet from disc C of experiment 20, a red and a green cornucopia were arranged side by side. Each contained honey.*

During the first five minutes that these cornucopias were watched, sixteen bees entered the red cornucopia and four the green.

EXPERIMENT 23.—*The green cornucopia of experiment 22 was replaced by a red cornucopia which did not contain, and never had contained, honey. This placed two red cornucopias side by side, one containing honey and the other empty.*

During the five minutes that these cornucopias were under observation, so many bees entered the red cornucopia which contained honey that it was impossible to count them; five entered the empty red cornucopia.

The empty red cornucopia was now placed where the one containing honey had been and the one containing honey placed in its stead.

During the first five minutes that they were observed, so many bees entered the cornucopia which contained honey that it was impossible to count them; many bees hovered around the empty cornucopia, but none entered.

The cornucopia that contained the honey was removed, the bees shaken out, and the cornucopia put out of sight. This left an empty red cornucopia in a part of the field which, for more than half an hour, had contained at least one red cornucopia which was well supplied with honey.

At first the bees circled around the cornucopia, presently one entered and then left immediately. Within ten minutes twenty-five bees had entered the cornucopia. (This does not mean twenty-five different bees, for the same bee entered more than once and was counted each time.) At first each bee left as soon as she had reached the inner depths of the cornucopia. Soon, however, the bees began to enter so rapidly and in such large numbers

that it was impossible for those that had reached the inner depths to leave without a struggle; and, in less than thirty minutes, the cornucopia was packed almost full of struggling bees, and numerous others were hovering around the mouth, seeking a place to enter.

EXPERIMENT 24.—*Ever since the beginning of this series of experiments, the red cornucopias on the melilotus had been kept well supplied with honey. At this time the weed contained eight red cornucopias and an equal number of empty green ones. Into the upper portion of this weed, I placed an empty red cornucopia.*

During the five minutes that this cornucopia was watched, twelve bees entered, one at a time, tarried a moment and then left.

EXPERIMENT 25.—*In the open, three feet from the empty red cornucopia of experiment 23, I placed, on a rod, an empty green cornucopia. In this place, earlier in the morning, there had been a red cornucopia well supplied with honey.*

During the ten minutes that this cornucopia was observed, many bees hovered around it; one alighted on the front platform, but none entered.

All of the cornucopias were removed from the field except the empty red cornucopia of experiment 23 and the empty green one of this experiment. This left only two cornucopias in the field; one red and one green, neither of which had ever contained honey.

This green cornucopia was watched continuously for ten minutes. During that time many bees hovered around the green cornucopia; two alighted on the front platform, and one entered. At the close of the ten minutes, I walked over to the empty red cornucopia and found it almost full of struggling bees, and numerous other bees were hovering around the entrance seeking admittance.

Series IV. (July 15, 3 P.M.)

This series of experiments was conducted with special cardboard boxes; each consisting of a rectangular outer case (8 × 5.2 × 2.5 cm.) with a porch-like extension in front and open ends, into which there was shoved, from the rear, a cardboard tray 5.5 × 5 × 2.4 cm. Near one side of the front end of this tray an entrance was made. In most cases this entrance was a rectangular opening 2 × 1.2 cm.; in a special case it was circular and 1.5

cm. in diameter. The tray was shoved in from the rear until its rear end was just inside of the rear edge of the outer case.

In constructing the outer case, a piece of cardboard was cut the shape and dimensions of figure two, folded along the dotted lines and glued where the sides overlap. In constructing the inner tray, a piece of cardboard was cut the shape and dimensions of figure three, folded along the dotted lines and glued, by the flaps, to the inner portions of the adjacent sides.

EXPERIMENT 26.—*In the same weed that has been the site of most of these experiments, I placed one green and two red boxes. The green box was placed near one of the red boxes. The red boxes contained honey, the green was empty.*

It had been raining all morning and it was still quite cloudy, and only a few bees were afield. About five minutes after the beginning of the experiment a bee noticed one of the red boxes. She examined it carefully from all sides, found the entrance

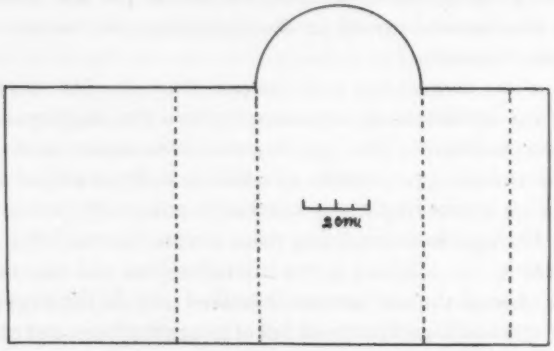


FIG. 2.

and entered. Soon after she entered, a heavy shower of rain began to fall and I took shelter under a tree. On my return (about fifteen minutes later) I found a large digger wasp in box number one and bees visiting red box number two. As long as that wasp remained in box number one, no bee would enter it. During the time that these boxes were under continuous observation, fifteen bees visited red box number one and twenty-eight visited red box number two. Towards the close of this experi-

ment, some of the bees would fly into the tray without first alighting on the portico. Not once was the green box visited by a bee.

EXPERIMENT 27.—*On my return after the rain mentioned in experiment 26, a watch-glass, seven centimeters in diameter, containing honey, was placed on the ground within a foot of the weed in which the boxes mentioned in experiment 26 were located. It was left uncovered; but, on such a cloudy afternoon, it was not conspicuous.*

During the time that I was watching experiment 26, no attention was paid to the watch-glass. At the close of that experiment the watch-glass was observed continuously for ten minutes. During that time not a single bee visited the watch-glass. Since the trip to the hive required less than five minutes, any bee that had succeeded in finding this watch-glass full of honey would have made at least one visit while I was watching; hence it is logical to conclude that no bee had visited it.

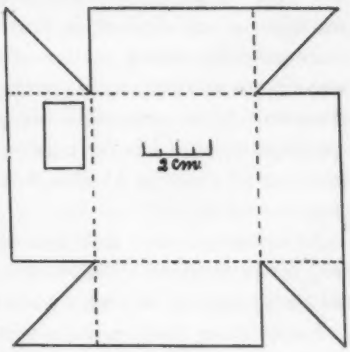


FIG. 3.

At this point rain caused a recess until eight A. M., July 16. On leaving for home, all of the boxes were removed from the weeds and the watch-glass taken from the ground.

EXPERIMENT 28.—*Side by side, in the same weed that has been the seat of the majority of these experiments, I placed two red experiment boxes; one contained honey and the other was empty.*

As soon as I appeared on the scene, the bees began to hover about me, and, before I could pin the red box with its supply of honey to the weed, a bee had entered its tray. In a few minutes so many bees were visiting the red box that contained the honey that it was impossible to count them. Frequently bees would enter the empty red box, but they would not tarry long.

EXPERIMENT 29.—*In the open space between the experiment weed*

and the hive, I arranged, on poles, about ten centimeters apart, three red boxes containing honey, one empty red box and one empty green box. The arrangement of the boxes in the group was altered once in ten minutes.

These boxes were under continuous observation for about an hour. Immediately the bees began to visit the boxes that contained honey in such large numbers that it was impossible to count them. Occasionally a bee would enter the empty red box and frequently they would hover in front of the entrance to its tray. No bee entered the green box, although occasionally a bee would alight on its top and pause long enough to clean its legs on an edge of it, and frequently one would pause a moment before some portion of the box. Whenever the honey was exhausted from one of the trays, the number of visitors would drop off. Seldom would a bee pass through the entrance; frequently one would hover momentarily before the door and then pass on. As soon as I had replenished the honey, the bees would begin to revisit it.

EXPERIMENT 30.—*After the above experiment had been under way for an hour, all of the boxes were removed from the field, except the empty red and the empty green box of experiment 29.*

Immediately the bees began to enter the red box more frequently than they had hitherto; as soon as one got well inside, it would leave. After a lapse of a few minutes, the bees began to rush for the entrance in such large numbers that those that had entered and wanted to leave could not do so without a struggle. As a result the tray and the portico were crowded with struggling bees, and numerous others were hovering about the entrance, seeking admittance.

At first no bees entered the green box, although many circled about it. After a lapse of ten minutes a few began to enter. During the period of observation, ten were noticed to enter it and leave immediately, and about twice that number were noticed to alight in the portico.

EXPERIMENT 31.—*Standing about three feet from the above boxes, I held, in my hand, a red box containing honey.*

Immediately a few bees approached and entered the box. I held the box in my hand for about five minutes. Throughout

that period there were from one to four bees inside of the box all the time.

EXPERIMENT 32.—*Standing in the same place mentioned in experiment 31, I held an empty red box in my hand. This box had never contained honey.*

Several bees approached and hovered around the box, and two entered.

INTERPRETATION OF THE EXPERIMENTS.

Vision or smell or some combination of the two are instrumental in guiding insects to flowers. Lately some observers have suggested that vision plays no part in this behavior; indeed, it is claimed that it is not even olfactory perceptions, but odors acting reflexly that lead insects to flowers. No one who has made observations for himself would think of claiming that smell plays no rôle in insect behavior; but, the experiments described above show conclusively that odors, acting reflexly, do not lead bees to flowers. In localities where bees are not accustomed to obtain honey from anything but flowers, honey may be placed on small discs (Ex. 1, 17), or in open vessels (Ex. 20–*A* and *B*, 27) and left exposed for a long time without being responded to by the bees. Bees, captured in bottles and turned loose upon discs that are well supplied with honey, will usually depart without paying any attention to the honey (Ex. 2). If a flower upon which such a bee is foraging is so manipulated as to bring the bee in close proximity to the honey of one of those discs, she will usually depart without responding to either the honey or the disc (Ex. 3, 4). It is incredible that an odor of honey too weak to attract bees a few millimeters off is able to entice them from several meters in the air. Then, too, each of my discs contained more than a thousand times as much honey as any one of those flowers did nectar, and the cornucopias that I used contained more than the discs; yet the bees passed by the feast of honey prepared for them to sip the meager supply of nectar stored in the neighboring small flowers. Such behavior would be impossible if their movements were controlled by the honey-odor acting reflexly. To claim that the nectar of the flower has a greater attractive power than the honey would be illogical for: 1st, honey is concentrated nectar; and 2nd, these same bees, after they have

learned to collect honey from objects other than flowers, will visit such objects as soon as they are attached to the support (Ex. 8, 19), at times they will even enter them while they are being attached to the support (Ex. 28), or they may even enter such an object while held in the hand (Ex. 31, 32).

If a set of bees has become accustomed to collect honey from artifacts, and paper discs, arranged in pairs of one red and one blue (or discs of any other two colors) are scattered on weeds or placed on weed-high rods, and honey is placed on the discs of one color and none placed on those of the other color, the bees will make regular visits to the color that bears the honey, but will not so respond to the other color (Ex. 8, 9, 10, 11). If these experiments are repeated, using cornucopias (Ex. 19) or boxes with small openings (Ex. 29) in place of the discs, the results will be the same. If bees have become accustomed to collect honey from an artifact of a certain color, and empty artifacts of the same kind and color are placed along side of those that contain honey, many of the bees will enter those artifacts that never have contained honey; empty artifacts of a different color are not responded to in that manner (Ex. 23, 24, 28, 29). After bees have, for a long time, been collecting honey from artifacts of a certain color, if all the artifacts be removed from the field except two that never have contained honey, but one of which is the color of the artifacts from which the bees have been collecting honey, numerous bees will flock into the empty artifact of the same color as those from which the bees have been foraging; but none, or nearly none, will visit the other artifact (Ex. 25, 30). When bees have become accustomed to collecting honey from other sources than flowers, if receptacles of two different colors are placed on a bush, all of one color containing honey and all of the other color being empty, and if, after the bees have been busy for a long time, honey is placed in one of the artifacts of the color that has been empty all along, it will remain on the bush some time before it will be visited by any bees (Ex. 12, 13, 21, 22). All of the facts recorded in this paragraph indicate that the behavior of foraging bees is influenced by colors.

That these bees not only respond to colors, but that they are capable of recognizing them at a distance is evidenced by the

following facts: (1) If, in an open space between their hive and a number of artifacts from which the bees are collecting honey, you place an artifact of the same kind and color and supply it with honey, it will be visited by bees almost immediately (Ex. 18, 29). (2) When bees are collecting honey from red artifacts situated in two different stations, one of which is much nearer the hive than the other and beneath the line of flight of the bees collecting from the more distant station, if all of the artifacts are removed from the more distant station, immediately the number of visitors to the other station is much increased (Ex. 18). This was the case even when the artifact in the nearer station did not contain honey (Ex. 30). Empty artifacts of another color than the honey-bearing color were not responded to in this manner (Ex. 30.).

On leaving one of these artifacts the bee usually made an orienting flight. On the first few visits this was thoroughly done. Facing the artifact and keeping about one centimeter from its surface, she would sidle, in a zigzag line, around the structure two or more times and occasionally reënter it one or more times. Then she would describe one or more spirals, pausing at certain places in the environment as though examining landmarks. In some of the cases I was, apparently, one of the objects thus scrutinized. In harmony with her other behavior, it seems plausbile to interpret this as an act by which memory pictures of the environment are formed.

How minute are the details that bees observe I am not prepared to say; but that they do observe details is indicated by the following observations. (1) In the boxes used in the experiments of Series IV., the trays of the boxes used were entered, from the portico, by means of an eccentric opening. In most of the boxes this doorway was rectangular, in others it was circular. When a bee first approached one of these boxes, she had to search for the entrance. After a few trips she would land on the portico directly in front of the entrance, and, in some cases, she would fly into the tray without even pausing on the portico. (2) For about an hour bees had been collecting honey from some red artifacts. It seemed that nearly all of the bees that visited that part of the field were collecting from those artifacts. Alongside

one of the red boxes I placed a red box on the sides and top of which I had pasted bits of white paper. This gave a box with red front and spotted sides. Into this box I placed some honey. The bees that approached this box from the front always entered immediately; the majority of those that approached the sides paused a moment, then went to the nearest red box.

Whether this is a true color vision or simply a greyness discrimination is no easy question to answer; indeed, from our viewpoint, it does not seem an important one. If what to us is red and green appears to the bees as two distinct sensations, as a factor for controlling behavior, it will have the same value to the bee whether it is a red-green discrimination or a grey-grey discrimination. However, the following line of reasoning has led me to believe this a case of true color-vision. Bees that had learned to respond to red boxes in the shadow of the weeds would respond, without hesitation, to similar boxes placed in the sunshine. They responded to the boxes when the sun was shining brightly just as readily as they did when a dark cloud hid the face of the sun. The brightness content of a body in the bright sunlight is quite unlike the brightness content of the same body when in the shadow of weeds; the brightness content of a body in the sunshine is quite unlike the brightness content of the same body beneath a cloudy sky. The only factor common to all of these cases is redness; hence I feel that, with the bees, it is a case of true color vision.

Although odor as a incitive to reflex actions does not play any part in leading bees to flowers, yet odor as a sensation does. If a large number of bees are collecting honey from a cluster of boxes that are all of the same color and you allow the honey of some of those boxes to become practically exhausted while that in the others is constantly replenished, when the workers approach the boxes that are practically exhausted, they, as a rule, do not pass inside; but, pausing momentarily before the box, pass on to one of those with an abundant store of honey. Replenish the empty tray with honey, and the next bee that approaches that box will enter (Ex. 29). This, to my mind, shows that when a bee approaches a box and finds the honey-odor weak she immediately departs for a box where the honey-odor is stronger.

These experiments prove that, to the bee, my colored discs, my colored cornucopias, and my colored boxes were something more than mere sensations; it seems to me that they were true percepts. To the bees those things had acquired a meaning; those strange red things had come to mean "honey-bearers," and those strange green things and strange blue things had come to mean "not-honey-bearers." Hence, whenever the bees saw the red things, they made the appropriate movements for securing the honey, and when they saw the blue things or the green things they passed on. This explains why, in the experiments of series one, discs six centimeters in diameter and well supplied with honey could remain in the presence of hundreds of bees without being responded to by them; and yet, those same bees, a few days later, when those things had acquired a meaning, would enter my red boxes even before I had had an opportunity to attach them to their supports. In their past experience those things had never acquired a meaning, while the small blossoms of the melilotus had come to mean "honey-bearers"; hence they hastened by the feast that had been prepared for them and rushed for the meager supply of nectar in the blossoms of the white sweet clover.

Although Plateau's conclusions are diametrically opposed to the results of this series of investigations, yet the facts related by him are in accord with them.

While proving that bees have color-vision, these experiments throw no light upon the color preferences of insects. That has not been the purpose of these researches. The aim has been to answer the question, Can bees distinguish colors? The experiments seem to demonstrate that foraging bees have percepts and that two factors which enter into those percepts are color sensations and olfactory sensations.

SUMMER HIGH SCHOOL, ST. LOUIS, MO.,
July 18, 1910.

BIOLOGICAL STUDIES ON CORYMORPHA. IV.[1]

Budding and Fission in Heteromorphic Pieces and the Control of Polarity.

HARRY BEAL TORREY.

The large solitary hydroid *Corymorpha* exhibits the phenomenon of heteromorphosis in forms even more striking than those under which it appears in the related *Tubularia*. At the same time, its normal polarity is in several respects more obviously marked. As against a stem, in *Tubularia*, that presents little or no indication of axial differentiation, the column of *Corymorpha* is divided into several regions sharply characterized by differences in form, structure and function. Its diameter varies, being greatest near the base, which is enveloped, for about one third the total length, in a thin layer of perisarc. Beyond the edge of the latter, the naked ectoderm is thicker, its cells are more narrowly columnar, and there is a marked increase in the number of nematocysts. Within the perisarc is the zone of frustules, or rootlets, that form the holdfast and have been homologized with the stolonal processes of *Tubularia*, although they are far more specialized structures. The proximal extremity, conical in form, is furnished with an amœboid ectoderm, by means of which the polyp creeps about.

Not only in structure does one find evidence of regional differentiation, but in capacity for regeneration as well. A hydranth is replaced after section of the column, with a velocity that decreases with the distance from the distal end of the intact hydroid. The differences in velocity are so slight as to be appreciated with difficulty in the distal half of the column, but are easily recognizable in a comparison of rates of regeneration in distal and proximal thirds. Furthermore, heteromorphosis,

[1]Contribution 32 from the Laboratory of the Marine Biological Association of San Diego. Preceding numbers of the Biological Studies on Corymorpha have appeared as follows: I., *C. palma* and Environment, *J. E. Z.*, 1 (1904), p. 395; II., The Development of *C. palma* from the Egg, *Univ. Calif. Publ. Zool.*, 3 (1907), p. 253; III., Regeneration of Hydranth and Holdfast, *ibid.*, 6 (1910), p. 205.

though it may occur after section of the column even below the frustular zone, in the extreme basal region, is most frequent in pieces cut from the column in its distal half.

At the very beginning of my observations on the regeneration of *Corymorpha*, I was struck with two facts: (1) that a segment from the distal half of the column and including the hydranth, does not develop a hydranth at the proximal end until the original hydranth is removed; (2) that when the original hydranth has been removed, the proximal hydranth develops, under normal conditions, more slowly than the distal—another indication, it may be mentioned, in passing, of the initial polarization of the column.

These facts suggested the possibility that, by delaying the development of the distal hydranth on a regenerating piece until the proximal hydranth should have reached an advanced stage of development, the initial polarization might be completely reversed. Accordingly, in the summer of 1902, I performed the following experiment.[1] A segment was cut from the distal half of an average polyp (Fig. 1, *A*). It was then inverted, and the distal cut surface held against the glass bottom of the aquarium by the weight of a steel needle through the distal region (Fig. 1, *B*). The proximal end was free and the stem vertical. At the end of three days, a hydranth flourished at *p*, though there was sign neither of tentacles nor frustules at *d*. The needle was removed. Two days later a small hydranth had appeared at *d* (Fig. 1, *C*), with three distal and four proximal tentacles, somewhat irregularly arranged, probably owing to the wound left by the needle and to other adverse conditions to which that end may have been subjected when pressed against the substratum.

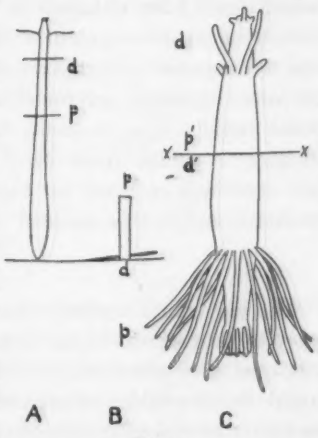


FIG. 1.

[1] All the regenerations noted in this paper occurred under starvation conditions.

It will be noted that while a distal hydranth failed to develop in contact with the substratum, it soon appeared when freed from this contact, in spite of the presence of the large promixal hydranth. On the supposition, however, that the result may not have fully indicated the real state of affairs in the heteromorphic segment, the latter was sectioned at the level x. In two days frustules were appearing at p'; the original polarity of this portion of the column was preserved. But frustules were also appearing at d', and two days later, were unmistakably defined. *In this latter region, therefore, the original polarity was reversed; on a segment of a given polyp, not only had hydranth appeared in the customary position of holdfast, but holdfast had appeared in the customary position of hydranth.*

The outcome of this experiment recalls the reversal of polarity which Loeb later obtained in *Tubularia crocea* when, after accelerating the development of the proximal hydranth by inhibiting the development of the distal, he cut a segment just distal to the proximal hydranth and found that a proximal was now produced more rapidly than a distal hydranth.[1] Morgan and Stevens obtained a similar result on *T. marina* although the polarity of the stem was reversed for but a very short distance from the proximal end in this species.[2]

II.

The suggestion, coming from the above experiment with *Corymorpha*, that section of the column between the hydranths merely disclosed a reversed polarity that already existed but was not, under the conditions, expressed in structural differentiation, led to a number of similar experiments which showed that the original result was in no sense exceptional. I will consider three series of these experiments.

In the first, nine heteromorphic pieces were sectioned at different levels to determine the extent to which each hydranth might control the intermediate region in regeneration. In no. 1, the distal hydranth was removed by a cut immediately below it

[1] *Pflüger's Arch.*, 102 (1904), p. 152; trans. in *Univ. Calif. Publ. Physiol.*, 1 (1904), p. 151.

[2] *J. E. Z.*, 1 (1904), p. 559.

(Fig. 2, *a*); it was replaced by another hydranth that at the end of ten days was in the condition shown in Fig. 2, *b*. No. 2 was a similar case. In neither of these cases was reversal exhibited. No. 3 died. No. 4 is represented in Fig. 3, the proximal hydranth being much less developed than the distal, and the plane of section passing near but not immediately distal to it. In four days both

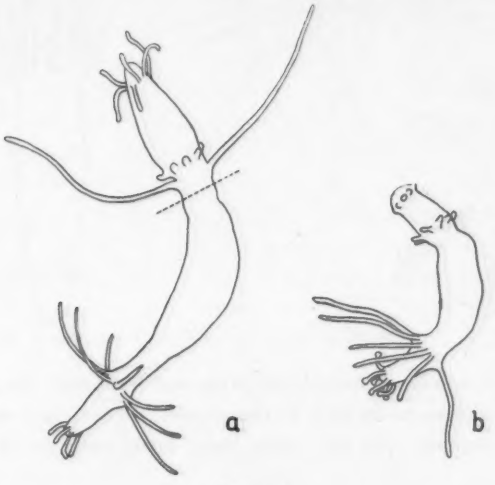


FIG. 2.

segments possessed frustules; the proximal segment had, accordingly, reversed completely. No. 5 (Fig. 4) exhibited another case of complete reversal in the proximal segment; both segments were as shown in Fig. 5 at the end of five days. No. 6 was a similar case. No. 7, cut when in the condition shown in Fig. 6, appeared, five days later, as shown in Fig. 7. No. 8, a similar case, exhibited similarly a complete reversal in five days. No. 9 resembled Fig. 2, *a*; but it was the smaller, *proximal* hydranth that was removed, by a cut immediately distal to it; in seven days a new hydranth was established in its place.

According to these results, heteromorphic pieces produce holdfasts at the wound when sectioned approximately midway between the hydranths, but produce hydranths at the wound on the longer pieces in those cases in which either hydranth has been removed by a cut immediately below it.

The second series of experiments involved 14 heteromorphic pieces whose proportions and stage of development are represented in the accompanying diagrams (Fig. 8). These pieces were cut as indicated in the diagrams. Four days later, both parts of *a*, *c*,

FIG. 3.

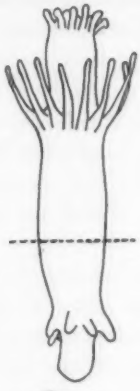


FIG. 4.

d, *e*, *f*, *h*, *i*, *l*, were attached and possessed frustules; the polarity of one of the pieces in each of these cases, accordingly, was completely reversed. At the same time, both portions of *b* were

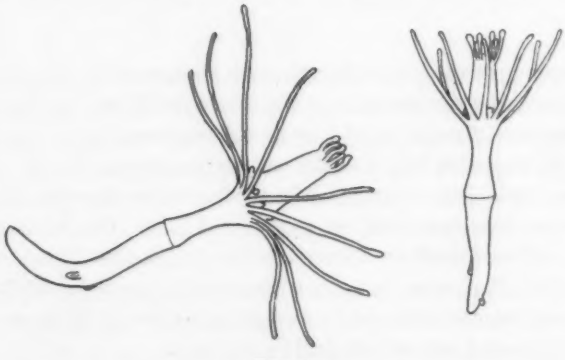


FIG. 5.

regenerating as single polyps, one being attached and furnished with frustules, the other being unattached and lacking frustules. Under *g*, four heteromorphic pieces were grouped. Four days

after cutting, all were regenerating as single polyps, six being attached and possessing frustules, two being unattached and lacking frustules. Both pieces of *k* were attached, three days after

FIG. 6.

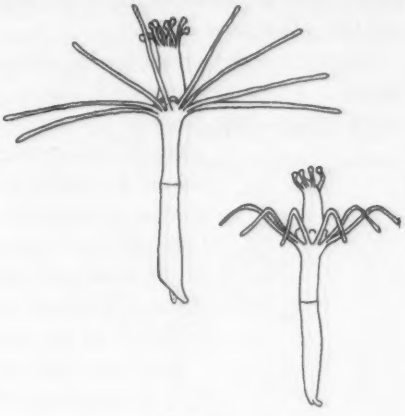


FIG. 7.

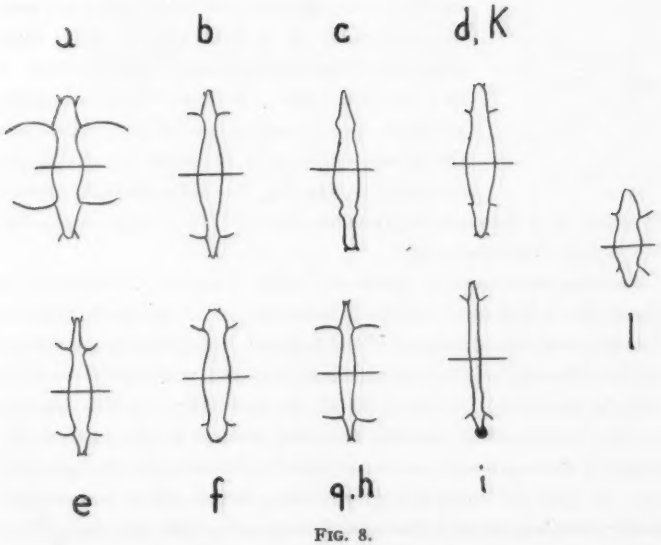


FIG. 8.

cutting, but owing to accidental neglect, disintegrated before forming frustules.

It should be noticed especially that all the heteromorphic pieces used in this series were *short*, and none were above medium diameter. *In not a single case, under these conditions, did the polarity fail to reverse in one of the portions into which the heteromorphic pieces were divided.*

The third series shows the importance of this consideration of length. Three heteromorphic pieces, absolutely and relatively much longer than those of the second series, one of them considerably larger than the other two, were sectioned as shown in Fig. 9. Five days after section, all six pieces were heteromorphic which indicated that in none of them, whether distal or proximal, was development at the wound dominated by the conditions, existing at the other end of the piece.

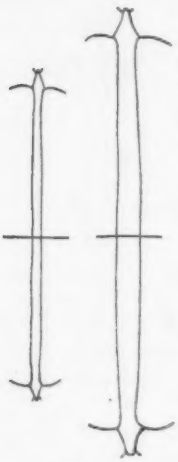


FIG. 9.

The same fact is brought into clear relief by a comparison of the following figures. Of 14 segments representing the distal half of the column of 14 polyps of moderate size, 12 were heteromorphic in 3 days. Of 81 very short segments from several small polyps, only 6, or 7.4 per cent., became heteromorphic. Further, of 13 segments of approximately the same length and diameter as the pieces obtained by cutting the heteromorphic pieces in series 2, 8 became heteromorphic. Of 15 similar segments, 10 became heteromorphic.

Besides these figures, there is a mass of evidence, obtained by repeated experiments on large numbers of individuals, demonstrating that the presence of the original hydranth on a segment of the column inhibits the development of a proximal hydranth.

It is clear, then, in the light of the facts cited in this section, (1) that *reversals of polarity profound enough to effect entire segments of the column as units are readily produced in Corymorpha;* and (2) that *the stage of differentiation at one end of a piece will under certain conditions control differentiation at the other end.* That reversals of polarity, in cases of heteromorphosis, are often shown, by form changes, to affect considerable areas of the column, without aid from the knife, will appear in the following section.

III.

As the first evidence in this direction I may refer at once to certain U-shaped figures formed from heteromorphic segments by the attachment of the latter to the bottom of the aquarium dish by means of adhesive ectoderm developed on one side of the column. Such a case is shown in Fig. 10, which represents the condition of a segment sixteen days after its removal from the distal half of a column. A constriction defines the aboral limits of the regenerating polyps. Frustules are present in two groups,

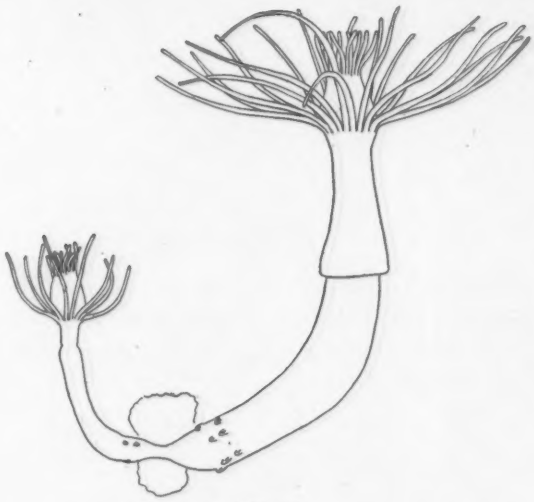


FIG. 10.

roughly proportional in numbers to the sizes of the polyps to which they belong, and situated in the position normally occupied by frustules in the adult. The smaller polyp approaches more closely to the proportions of the larva. On the following day, this process of fission had been completed, probably by rupture, although the proximal ends of the two polyps were then rounded and smooth without traces of such a process.

Through my failure, after repeated attempts, to obtain many cases of fission of this sort, I found that a necessary element in the process was the adherence of the heteromorphic piece to the

substratum. I pointed out some years ago[1] that *Corymorpha* is negatively geotropic, even small fractions of the column reacting with great definiteness. This tendency to bend away from the center of the earth can be effective, however, only when the reacting piece is properly anchored. Pieces free from the

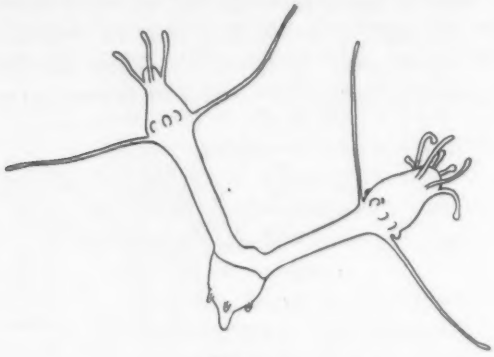


FIG. 11.

substratum never exhibit the reaction. As soon as they are attached, it appears; and U- and Y-shaped figures are formed when pieces are heteromorphic and the point of attachment is between the developing ends.

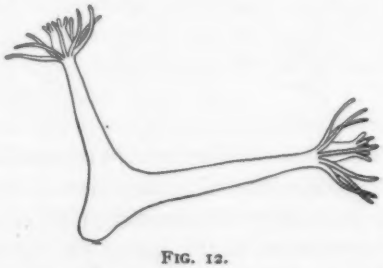


FIG. 12.

Each Y-shaped figure is formed from a straight piece by the development of a protuberance that corresponds to the stem of the Y, and is terminally adhesive (Figs. 11, 12, in both of which the limbs of the Y have become much attenuated during the

[1] *J. E. Z.*, 1 (1904), p. 395; *Univ. Calif. Publ. Zool.*, 2 (1905), p. 335.

development). Such protuberances and Y-shaped figures are found in nature only in the most exceptional cases, where they probably arise in response to the same conditions that bring them forth in the laboratory. To present these conditions with sufficient fullness, I must refer briefly to other abnormal forms that have often appeared in neglected aquaria.

It was often difficult, in warm weather, in the absence of running water, to prevent the growth of bacteria in the dishes in which freshly collected polyps were placed. Under the influence of fermentative changes induced by these conditions, the hydranths would cast their large proximal tentacles and medusiferous peduncles, the distal tentacles would be absorbed, and the column would come rapidly to be surmounted by a more or less rounded mass of tissue that might show two or three knobby irregularities. The columns were affected in a much less degree if at all. In fact it is quite easy to avoid these difficulties altogether by removing the hydranths as soon as the polyps are collected. This fact is doubtless due to the relatively large mass of protoplasm in, and the greater differentiation of the tissues of the hydranths.

Upon the reduction of the hydranths to the knobby masses just mentioned, and the removal of the débris composed of disintegrating tentacles and medusæ with their peduncles, the bacteria would disappear, fermentative processes would lead to the substitution, for the original hydranths, of various monstrous forms—double hydranths, hydranths with double or triple probosces in varying degrees of independence, combinations of hydranths with varying numbers of probosces, etc. These phenomena indicated the breaking up of the original single physiological system into several, the first sign of this multiplication appearing in the irregular form of the terminal mass of tissue. Each irregularity was the center of a budding process. And without laying any emphasis on the manner of its initiation, each budding process may be compared directly with the process by which the stem is produced in the Y-shaped figures we have been considering.

In one important respect these budding processes resemble each other; they stand, namely, for a certain disorganization (how

produced may, for the time, not detain us) in the original physiological system. In another respect they differ, in that they lead, in the one case, to a hydranth, or part of a hydranth, in the other to a holdfast. This difference is essentially an expression of the different conditions controlling their development, of which the influence of adjacent parts is the chief. The intimacy of this cöordination is obviously a function of the physiological isolation of the parts concerned. It is a conspicuous fact that Y figures are formed almost invariably from short heteromorphic segments; shrunken, starving, slow developing (Fig. 13, which shows eth beginning of a bud) pieces give an especially large proportion of them. Frequently that portion of a segment of the column which lies against the floor of the aquarium puts forth tentacles more slowly than the upper surface. This retardation in develop-

FIG. 13.

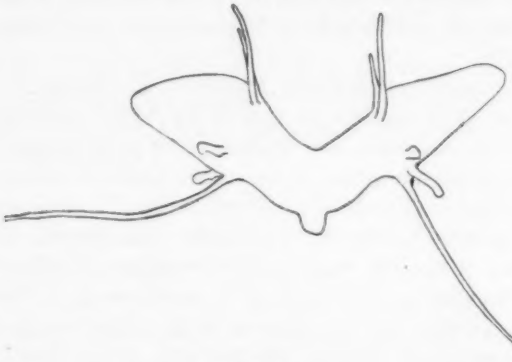


FIG. 14.

ment, due probably to diminished supply of oxygen next the substratum, is accompanied by a bending of the segment by means of a contraction of the affected side. It is on the opposite, convex, aspect of the column that the bud develops (Fig. 14, in which tentacles and gonads are only partly drawn), not, therefore, in direct contact with the substratum. And it is significant that, with the rarest exceptions that are referable to exceptional con-

ditions, such buds arising on heteromorphic pieces become holdfasts.

These facts indicate that while the origin of the bud depends upon a degree of disorganization in the original physiological system, its *fate* depends upon a secondary physiological coördination with the hydranths between which it develops. The bud acquires the distinctive character of a holdfast, namely, its adhesiveness, independent of any influence of the substratum. It has been pointed out already that a hydranth at one end of a piece exercises a profound influence upon the differentiation that may occur at the other end, depending on its own stage of development and its distance from that end. In the Y figures, then, there is a developing region between the two hydranths whose differentiation is controlled to some extent by them. An interesting case of the coördination of parts in a short piece developing as a Y figure, is represented in Fig. 15. A new axis at right angles to the original axis has been established. And the orientation of gonads and tentacles—especially the latter—is clearly a resultant of the redistribution of forces correlated with that change.

FIG. 15.

Fission may be considered as a special case of budding, depending upon the length of the heteromorphic piece involved. As the piece lengthens the tendency to bud vanishes, until the characteristic of adhesiveness alone remains. This indicates that the region between the two hydranths is still controlled by them, while the constriction and the frustules on either side of it (Fig. 10) mark the increasing effectiveness of their independence—or of the systems of which they form important parts.

It is only on heteromorphic pieces of moderate length, however, that fission of this type has been observed. Just as it was from relatively short heteromorphic pieces only that the reversals described in the previous section were obtained, the proximal elements of the longer pieces failing to produce holdfasts at the wound, so it has been only in the shorter heteromorphic pieces that the column between the hydranths has shown any signs of budding, constriction or frustule formation.

The longer pieces failed to differentiate in these directions, although attempts were made to encourage such developments, as follows:

1. Nine long heteromorphic segments were held against the aquarium bottom by weighted glass needles laid across their middle. One was cut in two in two days; neither portion had developed frustules twenty-four hours later. One was cut in two in three days; both portions had aboral processes, but no frustules developed in the following twenty-four hours. Four escaped from their needles on the third day, and showed no change at the end of another day. One was almost cut through by the needle, and three processes were formed at the wound, but no frustules or adhesiveness at the end of another day. One remained under the needle unchanged for four days, when the experiment terminated with my departure from the laboratory.

2. Discontinuity was produced by ligature on 16 long heteromorphic pieces two days after regeneration had begun. In two days, 3 had broken into two parts. Two days later, 6 more had done so; the next day, 7 more. Seven days after the ligatures were applied, 2 had frustules at the ligatures. Both were contracted and opaque—signs of structural degradation; 3 possessed no frustules at the ligature; on the contrary, the proximal segment of one possessed a hydranth there. All the other ligatured pieces had separated into two portions, 18 in all, of which but 4 were attached aborally and possessed frustules.

The first of these experiments serves to emphasize the feebleness of contact as a formative influence, while the second adds to the evidence that differentiation in the region between heteromorphic hydranths depends in an important degree upon the distance between them, which other things equal, is an index of their control.

That the longer pieces do not show signs of fission, then, is to be attributed, I believe, to the freedom of the intermediate region in each case from the effective control of the physiological systems on either side, that may be conceived as extending over it from opposite directions. Where these systems are near enough together, a new compound system is created, of which a bud may form a part. When farther apart, their disharmony may appear in the phenomnea of fiisson.

These statements are obviously very general. Since all short heteromorphic pieces do not either bud or divide by process of fission, there must be a factor still undefined that determines the lack of uniformity. There is no doubt that external agencies can facilitate either budding or fission. Small wounds in the side of the column may lead to a variety of results, including sporadic tentacles, and buds furnished with tentacles or frustules, or neither. In this connection two heteromophic pieces, cut in the middle region, half way through the column, gave the following results. On the longer piece, a narrow bud developed in four days at the wound, with neither frustules nor tentacles. On the shorter piece, frustules developed around a blunter bud at the wound, before attachment took place. In this case, the wound was sufficient to break up the original system existing at that point and initiate a new development. The fact that frustules appeared shows the control of both hydranths on that development.

Budding occurs, however, when there is no sign of local injury from without. And it is difficult to account on this ground alone for the fact that the large majority of buds arise approximately midway between the hydranths. To my mind, far more significant is the fact that buds develop so often on pieces obviously in poor physiological condition *generally*. It is then that the physiological continuity of the piece through the transitional middle region might be expected to be especially affected by disruptive tendencies springing from the antagonism of proximal and distal systems—so obviously antagonistic in the fission shown in Fig. 10. In that case, the canals are completely obliterated at the constriction and the tissue is opaque and apparently impoverished. This constricted region is under tension, which probably accounts in part for its form. The tension is produced by the active migration of the two polyps away from each other, in the manner of the opposite halves of an anemone in process of fission by rupture.[1] The initial discontinuity is thus accentuated by the activities of the polyps themselves.

[1]Torrey, *Univ. Calif. Publ. Zool.*, 1 (1904), p. 211.

IV.

Discontinuity can be established experimentally not only by the knife, which entails a wound, but, as already indicated, by ligature, by the use of which a wound can be avoided and conditions obtained that more nearly approximate those described in the last section.

This method has been used on *Tubularia* by Driesch, Morgan, Loeb, and Morgan and Stevens, and results obtained which are of interest in the present connection. By ligating segments of the stem, not only is the production of aboral (proximal) hydranths assured, but accelerated; and only exceptionally, after much longer periods, is there any development at the ligature itself. Loeb succeeded in showing that the acceleration of the development of the aboral hydranth is an indication of reversed polarity that exhibits a certain stability in regeneration. This is in accord with what I had already observed in *Corymorpha*, where reversals of polarity accomplished without the aid of the ligature are even more marked.

The experiments with ligatures have been repeated so many times on *Tubularia*, that it is hardly necessary for me to refer at present to similar experiments of my own farther than to say that the ligature accelerated the development of the aboral but not of the oral hydranth, and in no case was there any development at the ligature, on either side of it.

In Corymorpha, as in Tubularia, ligatures accelerate the velocity of development at the proximal ends of segments of the column. The fact does not stand out with such dramatic clearness, however, partly because there is greater individual variation in rate of regeneration, partly because the time intervening between the appearance of distal and proximal hydranths is much shorter. That such an acceleration occurs can be shown by an experiment like the following: Segments about 2 cm. long were cut from the distal half of 20 polyps, a ligature being passed tightly around each near its distal end. Segments of similar length were cut from the distal halves of 21 polyps of similar size; these were not ligatured. All were placed together in the same dish. In 28 hours, there were signs of proximal hydranths on 13 ligatured segments, and 14 on non-ligatured segments. The condition of

affairs at the end of 56 hours is shown in the following table, in which the serial numbers represent stages in the development arbitrarily selected for purposes of classification. Under each of these appears the number (1) of proximal hydranths in that stage of development on the ligatured segments, (2) of proximal hydranths on non-ligatured segments, and (3) of distal hydranths on non-ligatured segments.

TABLE I.

Dev. stages	1	2	3	4	5	6	7	Totals.
1. Lig. (pr.)	0	5	3	5	5	1	1	20
2. Non-lig. (pr.)	4	4	5	6	1	1	0	21
3. " (dist.)	3	2	8	4	1	2	1	21

In spite of the individual variation represented by these figures, they *show a tendency in the proximal ends to develop hydranths somewhat more rapidly on the ligatured segments, and almost as rapidly on the latter as the distal hydranths on non-ligatured segments.*

V.

Although *Corymorpha* responds proximally like *Tubularia* when segments of the column are ligatured as above, there is an important difference in its response at the ligature, namely, in the rapidity with which hydranths are formed immediately below it. In *Tubularia*, a hydranth very rarely appears immediately below the ligature, and then only after the lapse of many days. In *Corymorpha*, on the contrary, hydranths form readily and frequently in this position.

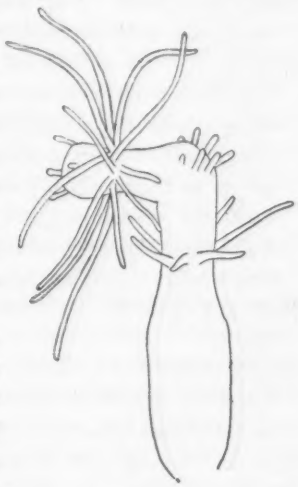


FIG. 16.

That a hydranth should arise immediately below a ligature in *Corymorpha* might be anticipated from the occasional occurrence of such monsters as that shown in Fig. 16, which represents a regenerating segment of the column. The proximal hydranth (below the angle) is not so far along as

the distal hydranth, and has apparently developed later, below an interruption in the physiological continuity of the column comparable with what a ligature might produce. In fact, just such cases have been produced experimentally several times. A typical experiment may be recorded, showing incidentally the difficulties that made the number of positive cases so small.

The column of *Corymorpha* is very mobile, capable of considerable changes in length and bulk, and its tissues are very delicate and easily ruptured. So it has been difficult to make ligatures tight enough to interrupt the currents in the canals, as well as possible diffusions through the axial cells, without so weakening the column as to lead to complete rupture in two or three days. This has been accomplished, however, in a number of cases sufficient for the present purpose.

EXPERIMENT 1.

April 29, 1910, 3.30 P. M. Sectioned 20 polyps of similar size, about midway of the column, and ligated each just below wound, leaving a small segment of tissue above the ligature.

May 1, 9.30 A. M. Four stumps removed, ligature having come away with terminal button of tissue.

May 2, 5 P. M. Thirteen more removed for similar reason. There are hydranths on these stumps that seem to be too far along, under the conditions, for 31½ hours (*i. e.*, assuming the separation to have occurred immediately after the previous survey of them, which is not probable).

Of the remaining 3, 2 show nothing below the ligature, while the third appears as in the semi-diagrammatic Fig. 17, which bears a striking resemblance to Fig. 16.

May 3, 9.45 A. M. The two hydranths of Fig. 17 have fallen apart.

May 4, 10.00 A. M. Of the 2 stumps showing no development below the ligature of May 2, one (*a*) has now budded a set of tentacles just below the ligature; the other (*b*) as before.

May 6. The tentacles of (*a*) are larger.

May 9. Still no change in (*b*). Exp. abandoned.

In a second experiment, 6 columns were tied as indicated in the diagram (Fig. 18, *a*), after removal of hydranth.

EXPERIMENT 2.

April 7, 3.00 P. M. Hydranths removed and columns tied.

April 8, 1.00 P. M. (*a*) Distal ligature and tip fragment (1) broken away in 2 cases. (*b*) The three segments have separated in 1 case. (*c*) 3 cases in original condition.

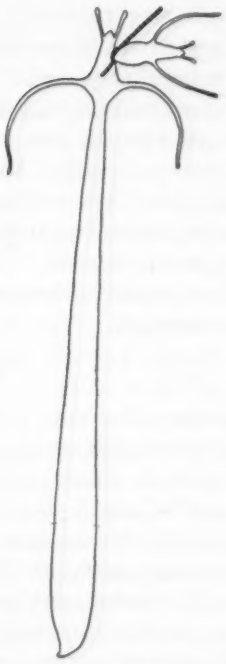


FIG. 17.

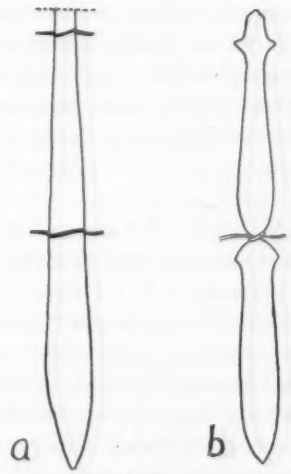


FIG. 18.

April 9, 2.00 P. M. (*a*) First case. Distal hydranth appearing on seg. 2, also on seg. 3 but not so far along. Second case. Distal hydranth appearing on seg. 2; not apparent on seg. 3, (*b*) —. (*c*) First case. The segments have separated while under observation. There are signs of a very slight rupture. and indications of tentacles on seg. 2 distally. Second case. The segments separated at 10.00 A. M. Seg. 2 shows signs of tentacles at both ends. In seg. 3, tentacles are being shadowed forth distally. Third case. The segments have separated,

seg. 2 showing faint signs of tentacles distally; seg. 3 in poor shape, removed.

April 10, 9.00 A. M. (*a*) First case. See Fig. 18, *b*. Note tentacles appearing just below ligature. Second case. Similar to first case; tentacles on seg. 3 not quite so far advanced.

April 11, 2.15 P. M. (*a*) Segments in both cases broken apart; exp. abandoned.

Of the six cases considered in this experiment, three show development of hydranths immediately below the ligature.

In a third experiment, the hydranth was removed from a polyp and the column ligatured near the cut and near the base. In three days, tentacles had budded just proximal to the distal ligature, as well as on the small distal segment. The next day, the latter was loosely joined to the segment proximal to it; both segments possessed hydranths with both sets of tentacles.

These results show that *hydranths form readily immediately below the ligature* in experiments like the foregoing.

VI.

The failure of *Tubularia* in such experiments to form a hydranth immediately below the ligature with the facility exhibited by *Corymorpha* is correlated with an important structural difference. The stem of *Tubularia* is encased in a stiff, chitinous layer of perisarc, that offers a certain barrier to the diffusion of gases between cœnosarc and the surrounding medium. The column of *Corymorpha*, furnished with a thin, rudimentary perisarc about its base, is naked for more than half its distal length; in this naked distal region, the ligatures were in all cases located.

When a ligature is passed tightly around a stem of *Tubularia*, the cœnosarc is not only ruptured, but the perisarc, itself intact in most cases, is drawn closely about each end of the cœnosarc thus produced. The result is that, while discontinuity has been established, the cœnosarc remains, as before, separated by the perisarc from the surrounding medium. When a ligature is passed tightly around a column of *Corymorpha*, discontinuity is established without in any way interfering in a comparable degree, if at all, with the diffusion of gases between cœnosarc and sea water. Now, when it is remembered that a discontinuity

brought about by a knife in *Tubularia* leads promptly to regeneration at the wound, the fact suggests that *Tubularia* develops with greater difficulty than *Cormyorpha* at the ligature because the cœnosarc receives a smaller supply of oxygen in that region.

This view was tested by the following experiment, in which the factor of contact, which tends to inhibit development orally in *Corymorpha*, was eliminated.

Fifteen pieces, each about 2 cm. long, were cut from fifteen *Tubularia* stems of similar size and condition, just below the hydranth. The distal end of each was inserted in a capillary glass tube (closed at one end by a paraffine plug) into which it fitted easily, without terminal contact, *except with a small quantity of sea water;* the proximal end was free. As a control, 16 similar pieces were cut, both ends remaining free.

Forty-five hours later, the pieces in the tubes had developed nine hydranths on the outer (proximal) ends, nothing on the inner ends. In the control, though fifteen of the sixteen pieces possessed distal hydranths, no proximal hydranths were visible.

Twenty-five hours later still, all the pieces in the tubes possessed proximal hydranths; nothing had developed on the inner (distal) ends. In the control, only eight proximal hydranths were present.

Removed now from the tubes, all the pieces rapidly produced normal hydranths distally.

This experiment seems to establish (1) *that the failure of Tubularia stems to form hydranths, when ligatured, immediately below the ligature, is due to lack of oxygen;* (2) *that encasing the cut distal end of a stem in a glass cap leads to the same acceleration of development of the proximal hydranth as does the presence of a ligature;* and (3) *that accordingly, such acceleration is due to the inhibition of the development of the distal hydranth in the absence of an adequate supply of oxygen, rather than to an interruption of or other change in the course of the circulation in the canals.* The circulation is merely a transportation system, carrying substances favoring development that are removed from the stream by those tissues especially that have free access to oxygen, that is, by the tissues at the open ends of the perisarcal tube. The possibility that oxygen might play such a role in this process of selection

was suggested by Loeb,[1] though in a somewhat different form in connection with a discussion of the function of the red pigment granules of the circulation.

The acceleration of proximal development in ligated pieces of *Corymorpha* is connected, not so much with any effect the ligature may have upon the supply of oxygen to the neighboring tissues, as with the inhibitory factors of contact and necrotic change that it introduces. The same factors may play a certain part in the inhibitory effect of the ligature in *Tubularia* also.

It will be remembered that if a piece of *Tubularia* or *Corymorpha* is ligated in the middle, development at the distal wound does not exhibit the acceleration characteristic of development at the proximal wound. This difference is to be explained, I believe, in the following way. Inhibition of development at the ligature on its proximal side hinders the utilization of a certain quantity of substances that would be used up proximally were the ligature not present. That the availability of this material for the distal end does not obviously accelerate the development distally is due, probably, to the initial acceleration of the distal over the proximal development in the absence of the ligature, that is, under conditions of active competition with the proximal end.

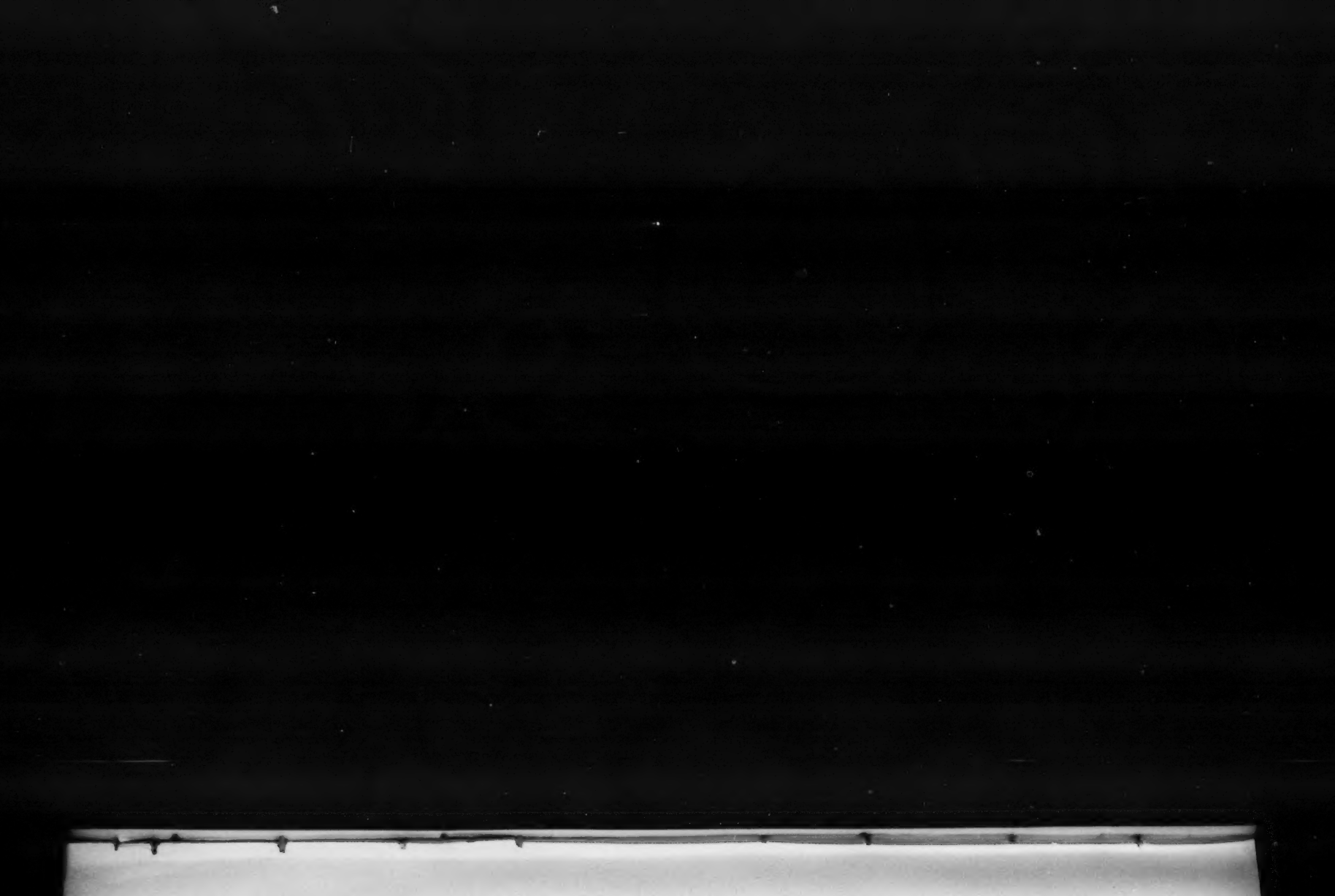
VII.

The considerations in the foregoing sections lead to the conclusion that the polarity of *Corymorpha*, of which the initial acceleration just mentioned is one expression, is a product of conditions under which the organism develops, changing as they change; that it is essentially but an inclusive designation for certain phenomena that depend upon both internal and external conditions, all of which can be experimentally controlled. The internal conditions appear in the effect of the continuity of tissue in an intact stem, and the presence of the original hydranth on a segment of stem, both inhibiting the development of a hydranth. The external conditions are represented by oxygen, contact, and necrotic changes such as are produced by ligatures. The first is necessary to all development while the others inhibit the development of the hydranth.

[1] *Loc. cit.*

With the exception of the third, these are conditions that govern the development, whether embyronic or regenerative, and they are just the conditions by whose manipulation the original polarity of the column can be reversed. Since such reversal depends upon an acceleration of proximal relative to distal development, it would seem reasonable to suspect that a local acceleration in the development of the embryo might be the efficient cause of the initial polarity in the individual.

Such a local growth area appears in the embryo at the point which first leaves the egg case and thereupon defines the oral extremity.[1] This extremity may emerge at any point not adherent to the substratum; which qualification indicates that contact limits to a considerable extent the area in which the oral pole must appear. Since the egg case is a certain barrier to the diffusion of oxygen into the egg it is possible that variation in its thickness may be an important factor in determining the position of the oral pole in this area.

ZOÖLOGICAL LABORATORY,
UNIVERSITY OF CALIFORNIA,
August 9, 1910.

[1]Torrey, *Univ. Calif. Publ. Zool.*, 3 (1907), p. 259.